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(54) Title: METHOD OF KILLING MICROORGANISMS

(57) Abstract

A method of killing microorganisms comprises the steps of mixing effective amounts of D-glucose and glucose oxidase, incubating the resulting mixture at a temperature of from -10 to 50 °C and at a pH of from 1 to 8 for a period of at least about 30 minutes and then applying the incubated mixture to the microorganisms.

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METHOD OF KILLING MICROORGANISMS

The present invention relates to a method of killing microorganisms such as bacteria, protozoa, fungi and viruses and to a packaged chemical composition 5 suitable for use in such a method.

International Patent Publication No. WO91/11105 (The Boots Company PLC) discloses anti-microbial compositions comprising iodide anions and thiocyanate anions in a weight : weight ratio of from 0.1:1 to 50:1 10 and having a combined anion weight concentration of at least 5 mg/kg, D-glucose in a weight concentration of at least 0.2 g/kg and an effective amount of the oxidoreductase enzyme glucose oxidase.

The present invention provides a method of killing 15 microorganisms by mixing effective amounts of D-glucose and glucose oxidase, incubating the resulting mixture at a temperature of from -10 to 50°C and at a pH of from 1 to 8 for a period of at least about 30 minutes and then applying the incubated mixture to the microorganisms to 20 be killed. Remarkably, the method of the invention provides improved speed of kill.

Preferably, the resulting mixture is incubated for at least 1 hour, preferably at least 2 hours, more preferably at least 4 hours. The inventors have found 25 that the fast-kill activity increases rapidly on incubation from 30 minutes to 48 hours.

A favoured incubation period is 12 to 48 hours. A particularly favoured incubation period is 24 hours, as the mixture can be made up a day in advance and the 30 incubation step carried out during transport to an end user.

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The incubated mixture of the invention has been found to retain its fast-kill properties for at least a 2 year test period.

5 Preferably, the concentration of glucose oxidase in the resulting mixture is at least 25 U/kg, suitably at least 150 U/kg.

Preferably, the resulting mixture comprises iodide anions and/or thiocyanate anions.

10 Suitably, the resulting mixture comprises both iodide anions and thiocyanate anions, suitably in a weight : weight ratio within the range 0.1:1 to 50:1 and suitably to a combined anion weight concentration of at least 5 mg/kg, preferably at least 10 mg/kg.

15 Iodide and thiocyanate anions are generally included in the compositions according to the invention in the form of salts. Suitable iodide salts include alkali metal salts such as potassium iodide and sodium iodide and mixtures thereof. Suitable thiocyanate salts include, for example, potassium, sodium, ammonium, 20 ferric and cuprous salts of thiocyanate and mixtures thereof. Preferably the weight concentration of iodide anions is at least 5 mg/kg and the weight concentration of thiocyanate anions is at least 2 mg/kg. The weight:weight ratio of iodide:thiocyanate anions is 25 preferably in the range 0.2:1 to 20:1, more preferably 0.5:1 to 15:1, particularly 1:1 to 5:1.

30 All units (U) of enzyme activity referred to herein relate to International Units of activity defined as the amount of enzyme required to catalyse the transformation of 1.0 micromole of substrate per minute at 25°C under optimal conditions. All concentrations referred to

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herein relate to amounts per kilogram of the total composition.

The method may be used to kill most if not all types of microorganisms, for example gram negative 5 bacteria such as Escherichia coli and Pseudomonas aeruginosa, gram positive bacteria such as Staphylococcus aureus and Propionibacterium acnes, moulds such as Aspergillus niger and Penicillium funiculosum, yeasts such as 10 Candida albicans, Saccharomyces cerevisiae and Pityrosporum ovale, dermatophytic fungi such as Trichophyton rubrum, microalgae such as Chlorella spp. and Spvrogryra spp. and viruses such as Herpes virus and Picornavirus.

15 The oxidoreductase enzyme, glucose oxidase, catalyses the production of H_2O_2 by oxidation of D-glucose in the presence of water and oxygen. It is classified as E.C.1.1.3.4. (IUPAC) and is defined herein in International Units (amount of enzyme required to 20 catalyze the oxidation of 1.0 micromole β -D-glucose per minute at pH 7.0 and 25°C). Glucose oxidase is available commercially from a number of sources, for example from Sturge-ABM under the trade designations "Glucox P200" (2000 U/ml) and "Glucox PS" (75 U/mg). 25 Glucose oxidase concentrations in excess of 150 U/kg provide excellent protection against bacterial, mould and yeast growth.

The oxidisable substrate for glucose oxidase, namely D-glucose, is generally included at a concentration (in the resulting mixture, before incubation) of at least 0.2 g/kg, preferably at least 0.5 g/kg, preferably at least 1 g/kg, and more particularly at least 2 g/kg. It will be appreciated by those skilled in the art that D-glucose may be provided per se or may

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be formed in situ from suitable precursors, for example, as a result of the breakdown of an oligomer or polymer containing D-glucose. Suitable precursors such as sucrose or starch may be used alone or in admixture 5 with D-glucose and may advantageously support more sustained anti-microbial activity than obtained with D-glucose alone.

The efficiency of iodide and thiocyanate anion oxidation in the presence of H_2O_2 may be enhanced by the 10 addition of small amounts of a peroxidase enzyme such as lactoperoxidase, preferably at least 100 U/kg lactoperoxidase. Lactoperoxidase is classified as E.C.1.17.1.7 (IUPAC) and is defined herein in International Units (amount of enzyme required to 15 catalyse the reduction of 1.0 micromole H_2O_2 per minute at pH 7.0 and 25°C). Lactoperoxidase is available commercially from a number of sources, for example from DMV (De Melkindustrie Veghel bv) (275 U/mg). It may be supplied, for example, in the form of a freeze-dried 20 powder or in an aqueous salt solution e.g. 1.8% NaCl or 12% NaCl. The compositions according to the invention which further comprise lactoperoxidase exhibit effective killing activity against the organisms listed above.

The method of the invention may, if desired, 25 incorporate further agents which may supplement or enhance the anti-microbial activity thereof, for example other enzymes such as lactoferrin or salts such as calcium chloride. Anti-microbial activity may be enhanced by the addition of agents having antioxidant 30 activity. Typical antioxidants include, for example, butylated hydroxyanisole, butylated hydroxytoluene, α -tocopherol and esters thereof, ascorbic acid, salts and esters thereof, gallic acid, salts and esters thereof e.g. propyl gallate, quinones such as 2,5- 35 ditertiary butylhydroquinone, propolis, flavenoid-

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containing materials such as quercetin, sulphur-containing materials such as dilauryl-3,3-thiodipropionate and distearyl-3,3-thiodipropionate, and mixtures thereof. Preferred antioxidants are selected
5 from butylated hydroxyanisole, butylated hydroxytoluene, α -tocopherol and esters thereof and ascorbic acid, salts and esters thereof, preferably in a weight concentration of at least 1 mg/kg, more preferably at least 50 mg/kg. The use of α -tocopherol and esters thereof as "natural" 10 antioxidants is particularly preferred.

There is also provided apparatus in the form of a packaged chemical composition for use in the method defined above, said apparatus comprising:

a) a first reservoir comprising a source of D-glucose;
15 b) a second reservoir comprising a source of glucose oxidase;
c) an incubation chamber connected to said first and second reservoirs; and
d) means for introducing controlled quantities of said 20 D-glucose and glucose oxidase into said incubation chamber to prepare and incubate a biocidal mixture of glucose and glucose oxidase ready for use.

Preferably, the first reservoir further comprises a source of thiocyanate and/or iodide anions.

25 Preferably, the second reservoir further comprises a source of lactoperoxidase.

Suitably, the effective concentrations of D-glucose, glucose oxidase, lactoperoxidase and thiocyanate and iodide are such as to provide a biocidal 30 mixture having the following composition on mixing (but before incubation):

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- (i) 10 to 500 mg/kg iodide anions;
- (ii) 5 to 200 mg/kg thiocyanate anions;
- (iii) 0.2 to 100 g/kg D-glucose; and
- (iv) 150 to 20000 U/kg glucose oxidase;

5 wherein the weight : weight ratio of iodide : thiocyanate anions is 0.2:1 to 20:1 and the combined anion weight concentration is at least 25 mg/kg, in combination with a suitable carrier or excipient.

One aspect of the invention provides concentrated 10 biphasic compositions in packaged and substantially non-reacting form which may be stored for prolonged periods prior to use. Concentrated compositions according to the invention will maintain physical separation of the glucose oxidase and its substrate, namely D-glucose, 15 such that H_2O_2 production is substantially prevented during storage. However, it will be understood that prior to storage concentrated compositions may contain a low level of at least one such substrate sufficient to support an initial reaction but insufficient to sustain 20 activity under the desired storage conditions. The initial reaction may advantageously provide adequate self-preservation of the concentrated compositions according to the invention. Self-preservation is of particular benefit in aqueous concentrates according to 25 the invention which may otherwise require the use of conventional chemical preservatives to avoid microbial spoilage during storage. The substantially non-reacting concentrated compositions according to the invention are intended to be mixed or diluted and activated 30 immediately prior to use by bringing the glucose oxidase and substrates thereof into intimate admixture to produce compositions having the desired anti-microbial properties.

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The concentrated compositions according to the invention optionally further comprise suitable carriers and/or excipients. Advantageously the compositions may incorporate at least one buffering agent to minimise the

5 fall of pH which may otherwise occur after activation of the concentrated composition. The concentrated compositions may be provided in the form of packs containing one or more discrete units of an appropriate weight or volume for batch or unit dosing.

10 Concentrated compositions according to the invention may comprise substantially anhydrous mixtures of each of the essential components mentioned hereinbefore, optionally combined with suitable non-aqueous carriers or excipients.

15 Concentrated water-containing compositions, optionally combined with suitable carriers or excipients, may be packaged and maintained prior to use. They may be in the form of, for example, solutions, suspensions, pastes or gels.

20 Compositions useful in the present invention may take the form of two or more powders, liquids, pastes or gels which prevent the glucose oxidase and D-glucose from reacting until the two are combined prior to use. For example, the glucose and D-glucose might be

25 formulated as dry granules to be activated by addition of liquid prior to use, allowing the reagents to react. Examples of such products include :

- a) deodorants e.g. for topical administration in the form of roll-on or stick formulations;
- 30 b) antibacterial skin washes e.g. in the form of lotions;
- c) anti-acne preparations e.g. in the form of lotions or creams;

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- d) anti-athlete's foot preparations e.g. in the form of lotions;
- e) anti-dandruff preparations e.g. in the form of shampoos or lotions;
- 5 f) dental preparations e.g. mouth washes suitable for general oral hygiene and in particular having anti-plaque properties, and dentifrices such as toothpastes, toothpowders, chewing gums and lozenges;
- 10 g) impregnated materials e.g. wound dressings, sutures and dental floss;
- h) pharmaceuticals e.g. wound irrigants and burn treatments, anti-diarrhoeal agents and medicaments suitable for the treatment of infections such as
- 15 15 Candida and Tinea infections;
- i) ophthalmic preparations e.g. eye washes and solutions for rinsing and/or sterilising contact lenses;
- j) sterilants e.g. for baby bottles and surgical or
- 20 20 dental instruments.
- k) sterilants for use in healthcare/manufacturing environments to decontaminate for example surgical instruments or food processing equipment; and
- l) surface cleaning agents for use in food
- 25 25 preparation/handling or healthcare environments to effectively decontaminate working surfaces.

In another aspect, it is preferred that the incubated mixture is provided as a concentrate for dilution to produce a solution for killing

30 30 microorganisms. In this aspect, the incubation step to activate the mixture for fast-kill activity is carried out during manufacture and/or distribution to an end user.

A range of oral hygiene preparations may be

35 35 envisaged which incorporate the anti-microbial

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compositions of the invention into conventional dental preparations such as mouthwashes, gargles and dentifrices as an anti-plaque agent and/or as a general antiseptic agent, for example in denture cleansing 5 tablets or solutions. The oral hygiene compositions of the present invention may, if desired, contain one or more active ingredients conventionally used in the art. These include, for example, other anti-plaque agents such as bromochlorophene, triclosan, cetylpyridinium 10 chloride and chlorhexidine salts; fluoride ion sources such as sodium fluoride, sodium monofluorophosphate and amine fluorides; anti-tartar agents such as zinc salts, preferably zinc citrate, and water soluble pyrophosphate salts, preferably alkali metal pyrophosphates; and 15 agents which reduce tooth sensitivity including potassium salts such as potassium nitrate and potassium chloride and strontium salts such as strontium chloride and strontium acetate.

It will be appreciated that the compositions and 20 methods of the invention are suitable for a whole host of anti-microbial applications in areas such as agriculture, horticulture, veterinary medicine, and aquaculture, such as trout farming.

The invention will be understood with reference to 25 the following non-limiting Tests and Examples:

Comparative Test A

Two phases of an antimicrobial composition were prepared as follows:

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Phase A

	<u>Component</u>	<u>Concentration (w/v%)</u>
	D-Glucose	45 to 55
	Sodium Thiocyanate	0.42 to 0.52
5	Potassium Iodide	0.66 to 0.80

Phase B

	<u>Component</u>	<u>Concentration</u>
	Lactoperoxidase	5500 Units/ml
	Glucose Oxidase	2250 Units/ml

10 Both phases were adjusted to between pH 5.5 and 6.5 with buffer solutions.

15 A test solution was prepared by mixing Phases A and B together with water to concentrations of 0.9% and 0.05% respectively. An aliquot of the resulting solution was removed immediately and inoculated with *Pseudomonas aeruginosa*.

20 The inoculated solution was incubated at room temperature. Samples were taken at regular intervals (viz at 0, 5, 10, 15, 30 and 60 minutes after mixing) and subjected to serial dilution and agar plating in known manner (normal aseptic technique being used throughout) to determine the numbers of any surviving organisms.

25 At each serial dilution point a parallel sample was taken, inoculated into a nutrient broth and incubated and then checked at an appropriate time for visual signs of growth. These parallel samples acted as so-called "broth controls" to check that the inoculation had been

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performed correctly and to check whether viable organisms were present which might be missed by the serial dilution process. These controls show up as counts of "less than 10" (<10) on the agar plates.

5 The original mixture of Phases A and B and water was stored at room temperature for three months. Aliquots were taken at regular intervals (viz at 4 hours, 24 hours, 48 hours, 7 days, 13 days, 21 days, 27 days, 2 months and 3 months) and inoculated with
10 Pseudomonas aeruginosa as described above to determine any change in the speed of kill.

Results are set out below in Table 1. In each case the "broth controls" are recorded as "+ve" or "-ve" above the corresponding plate counts.

15 Plate counts are given in "colony forming units per ml" (c.f.u. ml^{-1}). In each case "T" denotes the test solution and "C" a control (the buffer solution without Phases A and B above).

Comparative Test B

20 The procedure of Comparative Test A above was repeated with the modifications that the test and control solutions were inoculated with *Staphylococcus aureus* rather than *Pseudomonas aeruginosa* and that the storage times were varied. Results are set out in Table
25 2.

Comparative Test C

The procedure of Comparative Test A above was repeated with the modifications that the test and control solutions were inoculated with *Escherichia coli*

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rather than *Pseudomonas aeruginosa* and that the storage times were varied. Results are set out in Table 3.

Comparative Test D

5 The procedure of Comparative Test A above was repeated with the modifications that the test and control solutions were inoculated with *Enterobacter cloacae* rather than *Pseudomonas aeruginosa* and that the storage times were varied. Results are set out in Table 4.

10 Comparative Test E

The procedure of Comparative Test A above was repeated with the modification that the test and control solutions were inoculated with *Corynebacterium xerosis* rather than *Pseudomonas aeruginosa* and that the storage 15 times were varied. Results are set out in Table 5.

Comparative Test F

The procedure of Comparative Test A above was repeated with the modifications that the test and control solutions were inoculated with *Candida albicans* 20 rather than *Pseudomonas aeruginosa* and that the storage times were varied. Results are set out in Table 6.

Comparative Test G

The procedure of Comparative Test A above was repeated with the modifications that the test and 25 control solutions were inoculated with *Aspergillus niger* rather than *Pseudomonas aeruginosa* and that the storage times were varied. Results are set out in Table 7.

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Comparative Test H

The procedure of Comparative Test A above was repeated with the modifications that the test and control solutions were inoculated with *Micrococcus luteus* rather than *Pseudomonas aeruginosa* and that the storage times were varied. Results are set out in Table 8.

Comparative Test I

The procedure of Comparative Test A above was repeated with the modifications that the test and control solutions were inoculated with *Staphylococcus albus* rather than *Pseudomonas aeruginosa* and that the storage times were varied. Results are set out in Table 9.

15 Comparative Test J

The procedure of Comparative Test A above was repeated with the modifications that the test and control solutions were inoculated with *Pityrosporum orale* rather than *Pseudomonas aeruginosa* and that the storage times were varied. Results are set out in Table 10

Comparative Test K

The procedure of Comparative Test A above was repeated with the modifications that the test and control solutions were inoculated with *Streptococcus mutans* rather than *Pseudomonas aeruginosa* and that the storage times were varied. Results are set out in Table 11.

Table 1

Pseudomonas aeruginosa c.f.u. ml⁻¹

Storage time	T	Sampling time (minutes)				
		0	5	10	15	30
5 hours	+ve	+ve	+ve	+ve	-ve	-ve
	2.1x10 ⁶	1.1x10 ⁶	5.8x10 ⁴	<10	<10	<10
4 hours	+ve	+ve	-ve	-ve	-ve	-ve
	1.7x10 ⁶	<10	<10	<10	<10	<10
24 hours	+ve	-ve	-ve	-ve	-ve	-ve
	<10	<10	<10	<10	<10	<10
48 hours	+ve	-ve	-ve	-ve	-ve	-ve
	<10	<10	<10	<10	<10	<10
10	+ve	+ve	+ve	+ve	+ve	+ve
	2.3x10 ⁶					

Table 1 continued

Storage time		Sampling time (minutes)				
		0	5	10	15	30
7 days	T	+ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	C	1.3×10^6				2.2×10^6
13 days	T	+ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	C	2.6×10^6				2.0×10^6
21 days	T	+ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	C	3.6×10^6				3.2×10^6
27 days	T	+ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	C	3.1×10^6				3.0×10^6

Table 1 continued

		Sampling time (minutes)					
Storage time		0	5	10	15	30	60
		+ve	-ve	-ve	-ve	-ve	-ve
2 months	T	<10	<10	<10	<10	<10	<10
	C	3.3×10^6				7.8×10^6	
3 months	T	+ve	-ve	-ve	-ve	-ve	-ve
	C	6.0×10^6				7.0×10^6	

Table 2

Staphylococcus aureus c. f. u. ml-1

		Sampling time (minutes)					
Storage time		0	5	10	15	30	60
0 hours	T	2.7×10^6	2.7×10^6	2.2×10^6	2.2×10^6	2.5×10^5	+ve <10
	C	2.9×10^6					
4 hours	T	2.6×10^6	2.5×10^5	2.4×10^4	2.0×10^1	2.0×10^1	+ve <10
	C	4.7×10^6					
24 hours	T	2.8×10^6	2.4×10^3	2.0×10^1	2.0×10^1	2.0×10^1	+ve <10
	C	2.8×10^6					
48 hours	T	2.8×10^4	<10	<10	<10	<10	+ve <10
	C	4.1×10^6					

Table 2 continued

Storage time		Sampling time (minutes)				
		0	5	10	15	30
5 days	T	+ve 2.7x10 ⁶	+ve 3.2x10 ³	+ve <10	-ve <10	-ve <10
	C	3.2x10 ⁶				
7 days	T	+ve 6.3x10 ⁵	+ve 5.0x10 ¹	+ve 1.0x10 ¹	-ve 7.0x10 ¹	-ve <10
	C	3.9x10 ⁶				
14 days	T	+ve 1.2x10 ⁴	+ve <10	-ve <10	-ve <10	-ve <10
	C	3.2x10 ⁶				
28 days	T	+ve 6.6x10 ²	-ve <10	-ve <10	-ve <10	-ve <10
	C	4.1x10 ⁶				

Table 2 continued

		Sampling time (minutes)				
Storage time		0	5	10	15	30
2½ months	T	+ve	-ve	-ve	-ve	-ve
		1.7×10^4	<10	<10	<10	<10
	C	2.6×10^6				
4 months	T	+ve	+ve	+ve	-ve	-ve
		4.8×10^5	<10	<10	<10	<10
	C	4.4×10^6				1.6×10^6
5 months	T	+ve	+ve	+ve	+ve	+ve
		1.3×10^6	3.4×10^3	<10	<10	
	C	2.3×10^6				2.0×10^6

Table 3

Escherichia coli c.f.u. ml⁻¹

Storage time		Sampling time (minutes)					
		0	5	10	15	30	60
5 hours	T	5.6x10 ⁶	+ve	4.8x10 ⁶	+ve	5.5x10 ⁶	+ve
	C	4.2x10 ⁶				8.4x10 ⁴	<ve
4 hours	T	+ve	-ve	-ve	-ve	-ve	4.6x10 ⁶
	C	2.7x10 ⁶	<10	<10	<10	<10	<10
24 hours	T	+ve	-ve	-ve	-ve	-ve	6.8x10 ⁶
	C	2.6x10 ⁶					
48 hours	T	+ve	-ve	-ve	-ve	-ve	-ve
	C	4.7x10 ⁶	<10	<10	<10	<10	<10
10 hours	T	+ve	+ve	-ve	-ve	-ve	4.5x10 ⁶
	C	3.3x10 ⁵	<10	<10	<10	<10	<10
							3.4x10 ⁶

Table 3 continued

Storage time		Sampling time (minutes)					
		0	5	10	15	30	60
6 days	T	+ve	-ve	-ve	-ve	-ve	-ve
		<10	<10	<10	<10	<10	<10
C	4.8×10^6					4.0×10^6	
9 days	T	+ve	-ve	-ve	-ve	-ve	-ve
		<10	<10	<10	<10	<10	<10
C	3.2×10^6					5.2×10^6	
14 days	T	+ve	-ve	-ve	-ve	-ve	-ve
		<10	<10	<10	<10	<10	<10
C	3.1×10^6					3.5×10^6	
23 days	T	+ve	-ve	-ve	-ve	-ve	-ve
		<10	<10	<10	<10	<10	<10
C	3.3×10^6					3.6×10^6	

Table 3 continued

		Sampling time (minutes)					
Storage time		0	5	10	15	30	60
28 days	T	+ve <10	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	C	2.3×10^6				3.0×10^6	
2 months	T	+ve 3.7×10^4	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	C	4.9×10^6				2.6×10^6	
3 months	T	+ve <10	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	C	3.2×10^6				2.5×10^6	

Table 4

Enterobacter cloacae c.f.u. ml⁻¹

		Sampling time (minutes)					
Storage time		0	5	10	15	30	60
0 hours	T	2.4x10 ⁶	+ve	+ve	+ve	-ve	-ve
	C	5.7x10 ⁶	3.5x10 ⁶	1.1x10 ⁶	3.9x10 ⁶	<10	<10
4 hours	T	+ve	-ve	-ve	-ve	-ve	-ve
	C	4.4x10 ⁵	<10	<10	<10	<10	<10
24 hours	T	-ve	-ve	-ve	-ve	-ve	-ve
	C	4.6x10 ⁶	<10	<10	<10	<10	<10
48 hours	T	+ve	+ve	+ve	-ve	-ve	-ve
	C	2.4x10 ⁶	<10	<10	<10	<10	<10

Table 4 continued

Storage time		Sampling time (minutes)					
		0	5	10	15	30	60
3 days	T	+ve 6.8x10 ⁵	+ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	C	2.8x10 ⁶					
8 days	T	+ve <10	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	C	3.1x10 ⁶					
14 days	T	+ve <10	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	C	4.7x10 ⁶					
23 days	T	+ve	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	C	5.0x10 ⁶					

Table 4 continued

Storage time	Sampling time (minutes)				
	0	5	10	15	30
28 days	T +ve 1.0x10 ¹	-ve <10	-ve <10	-ve <10	-ve <10
	C 5.8x10 ⁶				4.2x10 ⁶
2 months	T +ve	-ve <10	-ve <10	-ve <10	-ve <10
	C 3.2x10 ⁶				3.4x10 ⁶
3 months	T +ve	-ve <10	-ve <10	-ve <10	-ve <10
	C 6.7x10 ⁶				4.2x10 ⁶

Corynebacterium xerosis c.f.u. ml⁻¹

Table 5

Storage time	T	Sampling time (minutes)				
		0	5	10	15	30
5 hours	T	+ve 1.4x10 ⁶	+ve 9.3x10 ⁴	+ve 1.2x10 ⁴	+ve 5.1x10 ³	+ve <10
	C	1.3x10 ⁶				+ve <10
4 hours	T	+ve 3.4x10 ⁴	+ve <10	+ve <10	+ve <10	+ve 1.1x10 ⁶
	C	8.7x10 ⁵				
24 hours	T	+ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	C	7.3x10 ⁵				3.5x10 ⁵
48 hours	T	+ve 2.7x10 ³	-ve <10	-ve <10	-ve <10	-ve <10
	C	6.3x10 ⁵				8.2x10 ⁵
10						

Table 5 continued

Storage time	Sampling time (minutes)					
	0	5	10	15	30	60
6 days	+ve	-ve	-ve	-ve	-ve	-ve
	<10	<10	<10	<10	<10	<10
C	7.6×10^5				9.4×10^5	
		-ve	-ve	-ve	-ve	-ve
9 days	+ve	-ve	-ve	-ve	-ve	-ve
	<10	<10	<10	<10	<10	<10
C	1.2×10^5				9.5×10^5	
		-ve	-ve	-ve	-ve	-ve
14 days	+ve	-ve	-ve	-ve	-ve	-ve
	<10	<10	<10	<10	<10	<10
C	6.1×10^6				4.2×10^6	
		-ve	-ve	-ve	-ve	-ve
23 days	+ve	-ve	-ve	-ve	-ve	-ve
	<10	<10	<10	<10	<10	<10
C	1.4×10^6				1.8×10^6	
		-ve	-ve	-ve	-ve	-ve

Table 5 continued

Storage time	Sampling time (minutes)	Sampling time (minutes)			
		0	5	10	15
28 days	T	+ve <10	-ve <10	-ve <10	-ve <10
	C	8.5×10^5			9.3×10^5
2 months	T	+ve <10	-ve <10	-ve <10	-ve <10
	C	1.9×10^6			8.2×10^5
3 months	T	+ve <10	-ve <10	-ve <10	-ve <10
	C	6.9×10^5			6.1×10^5

Candida albicans c.f.u. ml-1

Table 6

Storage time		Sampling time (minutes)					
		0	5	10	15	30	60
5 hours	T	+ve	+ve	+ve	+ve	+ve	+ve
	C	2.8×10^6	3.1×10^6	3.2×10^6	3.2×10^6	2.4×10^6	1.0×10^6
4 hours	T	+ve	+ve	+ve	+ve	+ve	+ve
	C	2.6×10^6	3.1×10^6	1.2×10^6	2.5×10^5	<10	<10
24 hours	T	+ve	+ve	+ve	-ve	-ve	-ve
	C	2.1×10^6	3.1×10^6	1.2×10^6	<10	<10	<10
48 hours	T	+ve	+ve	-ve	-ve	-ve	-ve
	C	2.5×10^6	2.0×10^2	<10	<10	<10	<10
10	T	+ve	-ve	-ve	-ve	-ve	-ve
	C	1.8×10^6	1.6×10^6	<10	<10	<10	<10

Table 6 continued

Storage time	T	Sampling time (minutes)					
		0	5	10	15	30	60
6 days	+ve	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	2.6×10^6						2.2×10^6
15 days	+ve	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	8.6×10^4						1.3×10^6
23 days	+ve	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	5.5×10^5						1.5×10^6
27 days	+ve	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	4.9×10^5						3.1×10^6

Table 6 continued

Storage time		Sampling time (minutes)				
		0	5	10	15	30
2 months	T	+ve	-ve	-ve	-ve	-ve
		6.0×10^4	<10	<10	<10	<10
3 months	C	5.9×10^6				6.6×10^6
	T	+ve	-ve	-ve	-ve	-ve
		2.5×10^5	<10	<10	<10	<10
	C	1.8×10^6				2.0×10^6

Table 7

Aspergillus niger c.f.u. ml⁻¹

Storage time		Sampling time (minutes)					
		0	5	10	15	30	60
5 hours	T	+ve	+ve	+ve	+ve	+ve	+ve
	C	1.2x10 ⁶	5.0x10 ⁶	1.6x10 ⁶	1.4x10 ⁶	1.0x10 ⁶	1.2x10 ⁶
5 hours	T	+ve	+ve	+ve	+ve	+ve	+ve
	C	2.2x10 ⁷					1.3x10 ⁶
24 hours	T	+ve	+ve	+ve	+ve	+ve	+ve
	C	1.2x10 ⁶	1.2x10 ⁶	1.3x10 ⁶	5.0x10 ⁵	9.0x10 ⁵	4.0x10 ¹
4 days	T	+ve	+ve	+ve	-ve	-ve	-ve
	C	3.7x10 ⁶	1.0x10 ¹	<10	<10	<10	<10
	T	-ve	-ve	-ve	-ve	-ve	-ve
	C	1.2x10 ⁵	<10	<10	<10	<10	<10
	T	-ve	-ve	-ve	-ve	-ve	-ve
	C	2.4x10 ⁶					1.4x10 ⁶

Table 7 continued

		Sampling time (minutes)						
Storage time		0	5	10	15	30	60	75
7 days	T	4.0x10 ⁶	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	C	1.2x10 ⁶						9.0x10 ⁵
14 days	T	1.1x10 ⁶	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	C	2.3x10 ⁶						1.9x10 ⁶
22 days	T	1.0x10 ⁵	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	C	4.0x10 ⁶						2.9x10 ⁶
26 days	T	1.0x10 ⁶	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10
	C	3.0x10 ⁶						2.6x10 ⁶

Table 7 continued

Storage time		Sampling time (minutes)					
		0	5	10	15	30	60
2 months	T	-ve	-ve	-ve	-ve	-ve	-ve
	C	3.0x10 ⁶	<10	<10	<10	<10	<10
3 months	T	-ve	-ve	-ve	-ve	-ve	-ve
	C	4.0x10 ⁶					
	T	-ve	-ve	-ve	-ve	-ve	-ve
	C	9.0x10 ⁵	<10	<10	<10	<10	<10
	T	-ve	-ve	-ve	-ve	-ve	-ve
	C	3.0x10 ⁶					

Table 8

Micrococcus luteus c.f.u. ml⁻¹

Storage time		Sampling time (minutes)					
		0	5	10	15	30	60
0 hours	T	+ve	+ve	+ve	+ve	+ve	+ve
	C	1.7x10 ⁵	1.8x10 ⁵	1.7x10 ⁵	1.3x10 ⁵	1.2x10 ⁵	2.2x10 ⁵
4 hours	T	+ve	+ve	+ve	+ve	+ve	+ve
	C	2.0x10 ⁵	8.0x10 ⁵	2.5x10 ⁴	7.5x10 ²	4.0x10 ¹	<10
24 hours	T	+ve	+ve	+ve	-ve	-ve	-ve
	C	1.2x10 ⁵					
48 hours	T	+ve	+ve	+ve	-ve	-ve	-ve
	C	1.5x10 ⁵	1.4x10 ⁴	3.8x10 ²	<10	<10	<10
10	T	+ve	+ve	+ve	-ve	-ve	-ve
	C	4.7x10 ⁵	1.4x10 ²	<10	<10	<10	<10

Table 8 continued

Storage time		Sampling time (minutes)					
		0	5	10	15	30	60
6 days	T	+ve	+ve	+ve	+ve	-ve	
	C	1.2×10^5	3.3×10^4	1.0×10^2	<10	<10	
13 days	T	+ve	+ve	+ve	+ve	1.3×10^5	
	C	1.6×10^5					
20 days	T	+ve	+ve	+ve	+ve	+ve	2.0×10^5
	C	1.8×10^5	<10	<10	<10	<10	
27 days	T	+ve	+ve	-ve	+ve	-ve	8.4×10^4
	C	2.2×10^5	<10	<10	<10	<10	
5	T	+ve	+ve	-ve	-ve	-ve	2.0×10^5
	C	2.5×10^4	<10	<10	<10	<10	

Table 9

Staphylococcus albus c.f.u. ml^{-1}

Storage time	Sampling time (minutes)						
		0	5	10	15	30	60
5 hours	T	+ve	+ve	+ve	+ve	+ve	+ve
	C	2.3×10^6	1.1×10^6	2.0×10^6	2.0×10^6	1.6×10^6	9.4×10^6
4 hours	T	+ve	+ve	+ve	+ve	-ve	-ve
	C	1.9×10^6	8.2×10^5	6.7×10^4	1.5×10^4	2.0×10^1	<10
24 hours	T	+ve	+ve	+ve	+ve	-ve	-ve
	C	2.4×10^6					2.0×10^6
48 hours	T	+ve	+ve	+ve	+ve	-ve	-ve
	C	2.0×10^6	2.0×10^5	1.8×10^4	<10	<10	<10
10 hours	T	+ve	-ve	-ve	-ve	-ve	-ve
	C	1.3×10^6	1.5×10^2	<10	<10	<10	<10
							1.6×10^6

Table 9 continued

Storage time		Sampling time (minutes)					
		0	5	10	15	30	60
6 days	T	+ve	+ve	+ve	-ve	-ve	
		2.1x10 ⁷	1.1x10 ⁵	8.7x10 ²	<10	<10	
	C	3.6x10 ⁶				1.8x10 ⁶	
13 days	T	+ve	-ve	-ve	-ve	-ve	
		2.8x10 ⁵	7.0x10 ¹	<10	<10	<10	
	C	1.9x10 ⁶				2.3x10 ⁶	
20 days	T	+ve	-ve	-ve	+ve	-ve	
		2.7x10 ⁵	<10	<10	<10	<10	
	C	2.3x10 ⁶				2.5x10 ⁶	
27 days	T	+ve	-ve	-ve	-ve	-ve	
		1.8x10 ⁵	<10	<10	<10	<10	
	C	9.6x10 ⁵				1.1x10 ⁶	

Table 10

Pityrosporum ovale c.f.u. ml^{-1}

Storage time	Sampling time (minutes)					
	0	5	10	15	30	60
0 hours	T	6.1×10^4	4.8×10^4	5.5×10^4	+ve	+ve
	C	4.7×10^4			3.7×10^4	4.3×10^4
4 hours	T	+ve	-ve	-ve	-ve	-ve
		5.1×10^3	4.0×10^1	<10	<10	<10
24 hours	T	+ve	-ve	-ve	-ve	-ve
		9.8×10^3	<10	<10	<10	<10
48 hours	T	-ve	-ve	-ve	-ve	-ve
		<10	<10	<10	<10	<10
10	T					
	C	8.0×10^4				5.4×10^4

Table 10 continued

Storage time		Sampling time (minutes)					
		0	5	10	15	30	60
8 days	T	+ve 1.1x10 ⁴	-ve <10	sampling interrupted	-ve <10	-ve <10	
	C	1.8x10 ⁵					
15 days	T	-ve <10	-ve <10	-ve <10	-ve <10	-ve <10	
	C	2.1x10 ³					2.0x10 ³
22 days	T	+ve 6.0x10 ¹	-ve <10	-ve <10	-ve <10	-ve <10	
	C	2.9x10 ⁵					2.2x10 ⁵
29 days	T	-ve 9.0x10 ¹	-ve <10	-ve <10	-ve <10	-ve <10	
	C	4.0x10 ⁵					3.9x10 ⁵

Streptococcus mutans c.f.u. ml⁻¹

Table 11

Storage time	Sampling time (minutes)						
		0	5	10	15	30	60
5 hours	T	+ve	+ve	+ve	+ve	+ve	+ve
	C	3.5x10 ⁵	4.7x10 ⁵	3.6x10 ⁵	3.4x10 ⁵	3.4x10 ⁵	3.9x10 ⁴
4 hours	T	+ve	+ve	+ve	-ve	+ve	2.0x10 ⁵
	C	3.8x10 ⁵	1.1x10 ⁵	3.1x10 ⁴	1.3x10 ⁴	<10	<10
24 hours	T	+ve	+ve	+ve	+ve	+ve	2.1x10 ⁵
	C	2.2x10 ⁵	2.1x10 ⁵	8.4x10 ²	5.0x10 ¹	<10	<10
48 hours	T	+ve	+ve	+ve	+ve	+ve	2.1x10 ⁵
	C	2.9x10 ⁵					
10 hours	T	+ve	+ve	-ve	-ve	-ve	-ve
	C	1.0x10 ⁶	<10	<10	<10	<10	<10
							1.0x10 ⁶

Table 11 continued

Storage time		Sampling time (minutes)					
		0	5	10	15	30	60
7 days	T	+ve	+ve	+ve	+ve	-ve	-ve
	C	1.4×10^5	2.0×10^3	<10	<10	<10	<10
13 days	T	+ve	-ve	-ve	-ve	-ve	-ve
	C	1.7×10^5				1.3×10^5	3.1×10^5

EFFECT OF pH

The following tests were carried out to assess the effect of pH on anti-microbial activity.

Test Procedure

5 Two phases of an antimicrobial composition were prepared as follows:

Phase A

	<u>Component</u>	<u>Concentration (w/v %)</u>
	D-glucose	45 to 55
10	Sodium thiocyanate	0.42 to 0.52
	Potassium iodide	0.66 to 0.80

Phase B

	<u>Component</u>	<u>Concentration</u>
	Lactoperoxidase	5,500 Units/ml
15	Glucose Oxidase	2,250 Units/ml

Both phases were adjusted to between pH 5.5 and 6.5 with buffer solutions.

Test solutions were prepared by mixing Phases A and B together with citrate phosphate buffer at pH 3, 4, 5, 20 6, 7, 8 to concentrations of 0.9% and 0.05% respectively. The test solutions were then stored at room temperature for 48 hours.

Aliquots of the stored test solutions were removed and inoculated with a single organism from the following 25 organisms: E.coli, S.aureus, C.albicans, A.niger.

The inoculated solution was incubated at room temperature for 15 minutes serially diluted and agar

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plated to determine the numbers of any surviving organisms.

		Organism cfu ml ⁻¹			
		E.coli	S.aureus	C.albicans	A.niger
5	Initial Inoculum count	8.2×10^5	2.4×10^6	1.2×10^6	1.0×10^5
	pH 3	<10	<10	1.9×10^6	<10
	pH 4	<10	<10	<10	<10
	pH 5	<10	5.0×10^3	<10	<10
10	pH 6	<10	5.0×10^5	<10	<10
	pH 7	1.2×10^2	6.4×10^5	<10	3.0×10^1
	pH 8	<10	2.1×10^6	1.7×10^5	1.7×10^3

15 The above results demonstrate that pH can be chosen so as to kill a particular micro-organism selectively. For example, at pH 8 only E.coli is killed. Broad spectrum anti-microbial activity is obtained when the pH is around 4 in the above tests.

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Example 1Glycol paint for athlete's foot or acne

A biphasic glycol paint is prepared to the following composition:

	<u>Component A</u>	<u>Amount/50g</u>
5	Propylene glycol	30g
	D-Glucose	1g
	Sodium thiocyanate	8.4mg
	Potassium iodide	13.2mg
10	Water	to 50g

Component B Amount/50g

15	Glucose oxidase (available under the Trade Designation "Glucox P200")	112U 15ppm)
	Lactoperoxidase	275U (10ppm)
	Water	to 50g

Using a suitably designed dispenser 1 part of Component A is combined with 1 part of Component B and allowed to stand for the required activation time. After this period the mixed components can be applied to control two strains of inter alia *Staphylococcus aureus* and also *Propionibacter acnes*, *Candida albicans*, *Trichoderma rubrum*, *Trichoderma mentagrophytes* and *Trichoderma interdigitale*.

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Example 2Roll-on Deodorant

A biphasic roll-on deodorant is prepared to the following composition:

	<u>Component A</u>	<u>Amount / 80g</u>
	Tetrasodium EDTA	0.125g
	Mixture of stearates (available under the Trade Designation "Cithrol GMS A/S")	
10	Ethoxylated fatty alcohol (available under the Trade Designation "Cromul EM 0685")	3.75g
	Light liquid paraffin	3.75g
	D-Glucose	0.625g
	Sodium thiocyanate	5.25mg
15	Potassium iodide	8.25mg
	Water	to 80g

	<u>Component B</u>	<u>Amount / 20g</u>
20	Glucose oxidase (available under the Trade Designation "Glucox P200")	280U (37.5ppm)
	Lactoperoxidase	687.5U (25ppm)
	Water	to 20g

Using a suitably designed dispenser, 4 parts of Component A are combined with 1 part of Component B in a mixing chamber and allowed to stand for the required activation time. After this period the mixed components can be applied to provide the necessary killing activity against inter alia two strains of *Staphylococcus aureus*.

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Example 3Anti-dandruff shampoo

A biphasic antibacterial anti-dandruff shampoo is prepared to the following composition:

	<u>Component A</u>	<u>Amount/80gms</u>
5	Sodium laureth-2-sulphate (23% soln)	68.75g
	Zinc sulphate	0.125g
	Mixture of diethanolamides (available under the Trade Designation "Empilan CDE")	6.25g
10	Stearic acid toilet	1.25g
	Mixture of mono and distearates (available under the Trade Designation "Empilan EGMS")	3.75g
	D-Glucose	0.625g
	Sodium thiocyanate	5.25mg
15	Potassium iodide	8.25mg
	Water	to 100g

	<u>Component B</u>	<u>Amount/20gms</u>
20	Glucose oxidase (available under the Trade Designation "Glucox P200")	280U (37.5ppm)
	Lactoperoxidase	687.5U (25ppm)
	Water	to 20g

Using a suitably designed dispenser 4 parts of Component A are combined with 1 part of Component B and allowed to stand for the required activation time. After this period the mixed components can be applied to achieve the desired killing activity against inter alia *S. aureus* and *Pityrosporum ovale*.

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CONCENTRATED FORMULATION

	<u>Example</u>	<u>Sterilising Concentrate</u> <u>for Dilution</u>	Concentration
5	Glucose		45% w/v
	Sodium Thiocyanate		0.42% w/v
	Potassium Iodide		0.66% w/v
	Potassium Dihydrogen Phosphate		0.62% w/v
	Sodium Hydroxide		0.02% w/v
10	Glucose Oxidase		5600 U
	Lactoperoxidase		13750 U
	Water	q.s to	100% volume

15 In the above concentrated formulation the components are pre-activated by incubation during manufacture and distribution. The concentration is then diluted 1 part in 100 parts of water to yield a sterilising solution with fast-kill activity.

20 The concentrated formulation is advantageous because the weight of product is greatly reduced compared to the liquid solution, making the concentrated product relatively cheap to distribute. The concentrate formulation is also convenient for the end user because it is easier to transport and takes up less storage space than the equivalent liquid solution.

Example 4

Sterilant solution (eg for contact lenses)

A biphasic sterilant solution is prepared to the following composition:

5	<u>Component A</u>	<u>Amount/50g</u>
	D-Glucose	1g
	Sodium thiocyanate	8.4mg
	Potassium iodide	13.2mg
	Water	to 50g
10	<u>Component B</u>	<u>Amount/50g</u>
	Glucose oxidase (available under the Trade Designation "Glucox P200")	112U (15ppm)
15	Lactoperoxidase	275U (10ppm)
	Water	to 50g

Using a suitably designed dispenser 1 part of Component A is combined with 1 part of Component B and allowed to stand for the required activation time. After this period the mixed components can be applied to give effective control of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus mutans*, *Enterobacter cloacae* and *Candida albicans*.

An example of packaging suitable for use in the
method of the present invention will now be described,
by way of example only, with reference to the
accompanying drawings, in which:

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Figure 1 shows a side section view of a packaged chemical composition according to the present invention, along the line I-I of Figure 2; and

5 Figure 2 shows a plan section view of the packaged chemical composition of Figure 1 along the line II-II of Figure 1.

The packaged chemical composition, generally indicated as 1, comprises a first reservoir 2 and a second reservoir 3. The reservoirs are of approximately 10 equal size and are generally tubular in shape. The reservoir walls 4,5 are made from resilient polyethylene but it will be appreciated that other resilient materials, such as polyvinylchloride (PVC) or polyethyleneterephthalate (PET) may also be used if 15 desired. The walls are strong enough to avoid the risk of breakage but flexible enough to permit "squeezing" of the contents in use.

One of the reservoirs, 2, houses Component A from Example 1 above and the other reservoir, 3, houses 20 Component B therefrom. The reservoirs 2,3 are substantially sealed so that no leakage of contents can occur from one to the other. For convenience they are welded together at one point by a polythene web 6 which is continuous with the reservoir walls 4,5. The walls 25 of the reservoirs are independently deformable such that it is possible to deform one reservoir, for example by "squeezing", without deforming the other, if desired.

The lower end of the reservoirs provide a base 7 to allow the packaging to be rested in an upright position. 30 The upper ends of the reservoirs 2,3 are constricted to form a neck 8 which is blocked by a neck-piece 9 made of stiff non-resilient polyethylene. The neck 8 and neck-piece 9 support a bulbous incubation chamber 10. The incubation chamber 10 is constricted at its upper end to

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provide a second neck 11 which is threaded in the manner of a screw-top bottle neck to connect with a screw-cap 12 which may be used to close off the packaging when not in use.

5 Polythene tubes 13,14 extend substantially from the bases of the two reservoirs 2,3 respectively, through holes in the neck-piece 9, with which they form an interference fit, to the base of the incubation chamber 10. The tubes 13,14 are narrow enough to prevent
10 significant leakage of the contents of the reservoirs 2,3 into the incubation chamber 10 during normal storage. Such leakage as may occur is contained by the cap 12. However, when the cap 12 is removed and one or both of the reservoir walls is deformed, for example by
15 "squeezing", a portion of the reservoir contents is forced up the tube 13 or 14 by the pressure created and into the incubation chamber 10.

In use, controlled mixing of the reservoir contents (Components A and B of Example 1 above) is achieved by
20 squeezing each of the reservoirs in turn and/or simultaneously by roughly equal amounts to introduce equal quantities of the reservoir solutions into the incubation chamber 10. Indicia 15 are provided on the walls of the incubation chamber 10 to assist in
25 measuring the quantities to be mixed. The walls of the incubation chamber are made from transport/translucent polyethylene to assist in the measuring process but this is not essential and it will be appreciated that opaque plastics materials may be used if desired. The cap 12
30 may then be replaced and the apparatus 1 may be shaken gently to complete the mixing process. The resulting mixture is left to incubate for the desired period, typically from 5 to 10 minutes.

After incubation the cap 12 is removed and the
35 incubated anti-microbial mixture is used in the desired

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manner, by application to the feet or other areas affected by so-called "athlete's foot".

It will be appreciated that the packaging could house any of the other Examples given above and/or 5 numerous variants thereof. Where the components are not mixed in equal proportions (eg Examples 2 and 3 above) the reservoirs may be made of correspondingly unequal size and corresponding modifications may be made to the other parts of the apparatus as appropriate. Thus, if 10 four parts of component A are needed to each part of component B, then the reservoir housing component A may be squeezed four times as often as the reservoir housing component B (as in Example 3 above), until the desired quantity of mixture has been generated in the incubation 15 chamber 10. Alternatively or additionally, the indicia 15 may be used to measure the required proportions.

Instructions for mixing the components in the appropriate proportions will normally be supplied with the apparatus, preferably by printing onto the packaging 20 itself.

CLAIMS

- 1 A method of killing microorganisms by mixing effective amounts of D-glucose and glucose oxidase, incubating the resulting mixture at a temperature of 5 from -10 to 50°C and at a pH of from 1 to 8 for a period of at least about 30 minutes and then applying the incubated mixture to the microorganisms to be killed.
- 2 A method as claimed in Claim 1 wherein the resulting mixture is incubated for 12 to 48 hours.
- 10 3 A method as claimed in any preceding claim wherein the concentration of glucose oxidase in the resulting mixture is at least 25 U/kg.
- 15 4 A method as claimed in any preceding claim wherein the resulting mixture comprises iodide anions and/or thiocyanate anions.
- 5 A method as claimed in any preceding claim wherein the resulting mixture comprises both iodide anions and thiocyanate anions.
- 20 6 A method as claimed in Claim 5 wherein the weight ratio of iodide anions to thiocyanate anions is from 0.1:1 to 50:1.
- 7 A method as claimed in Claim 6 wherein the combined anion weight concentration is at least 5 mg/kg.
- 25 8 A method as claimed in any preceding claim wherein the resulting mixture further comprises a peroxidase enzyme.
- 9 A method as claimed in Claim 8 wherein the peroxidase enzyme is lactoperoxidase.

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10 A method as claimed in any preceding claim wherein the incubated mixture is provided as a concentrate for dilution to produce a solution for killing microorganisms.

5 11 A method as claimed in any of Claims 1 to 9 wherein the components are provided as a concentrated biphasic composition in packaged and substantially non-reacting form.

10 12 Apparatus in the form of a packaged chemical composition for use in the method defined in Claim 1 said apparatus comprising:

- a) a first reservoir comprising a source of D-glucose;
- b) a second reservoir comprising a source of glucose oxidase;
- 15 c) an incubation chamber connected to said first and second reservoirs; and
- d) means for introducing controlled quantities of said D-glucose and glucose oxidase into said incubation chamber to prepare and incubate a biocidal mixture

20 of glucose and glucose oxidase ready for use.

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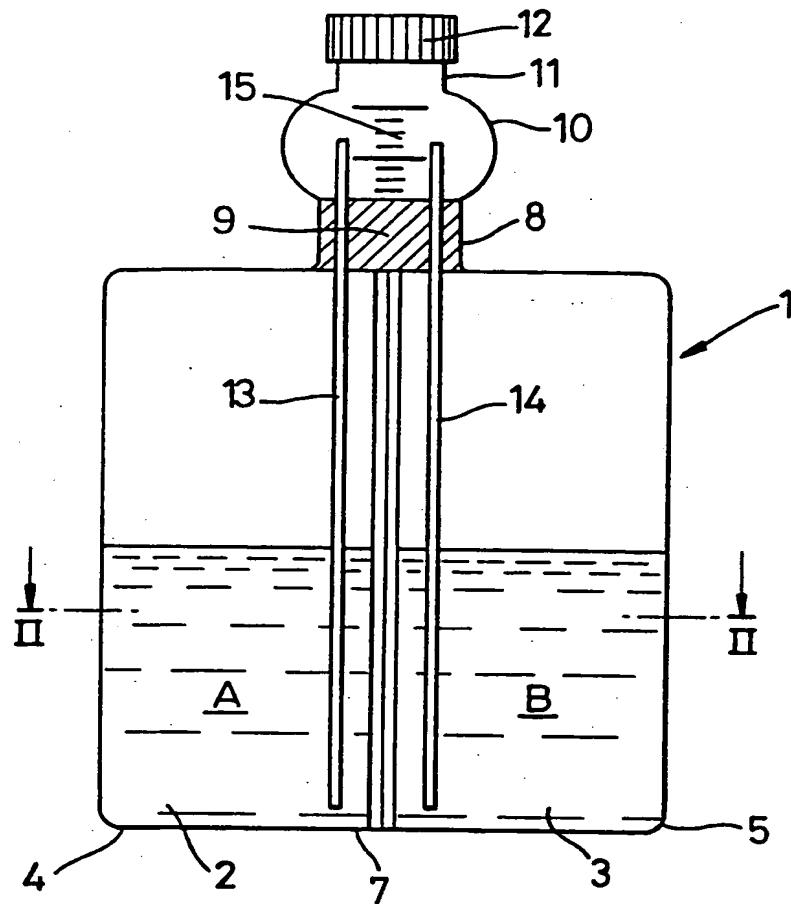


Fig. 1

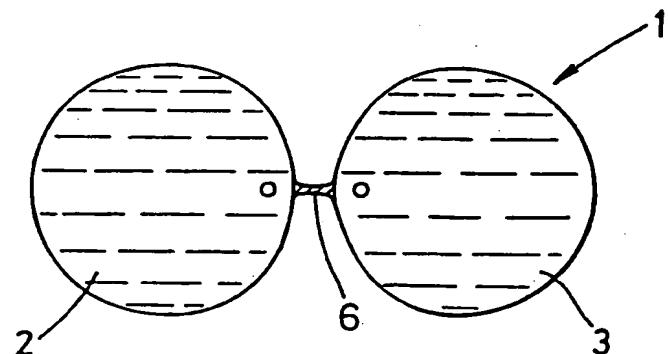


Fig. 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/01037

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01N63/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,91 11105 (THE BOOTS COMPANY) 8 August 1991 cited in the application see claims see page 6, column 28 - page 8, column 29 -----	1-12

<input type="checkbox"/>	Further documents are listed in the continuation of box C.	<input checked="" type="checkbox"/>	Patent family members are listed in annex.
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- *'A' document defining the general state of the art which is not considered to be of particular relevance
- *'E' earlier document but published on or after the international filing date
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- *'O' document referring to an oral disclosure, use, exhibition or other means
- *'P' document published prior to the international filing date but later than the priority date claimed

- *'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- *'&' document member of the same patent family

Date of the actual completion of the international search 26 June 1995	Date of mailing of the international search report 30.06.95
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+ 31-70) 340-3016	Authorized officer Decorte, D

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 95/01037

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